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Diluted Thermopressurized Phosphoric Acid: A Gentle Proton Donor for Polysaccharide Acid Depolymerization and (Bio)processing

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68236>

Abstract

Phosphorus is a very important element for several metabolic pathways in all living organisms as exemplified by DNA, RNA, glucose and fructose-P, and adenosine triphosphate (ATP). The whole metabolism of phosphate in any living organism involves the catalysis carried out by many enzymes, such as kinases, pyrophosphorylases, isomerases, and phosphatases. Symptoms of hypophosphatemia include neurological dysfunction and disruption of muscle and blood cells and could be caused by malnutrition, failure to absorb phosphate, and metabolic syndromes. Phosphoric acid is widely used as an acidifying agent in a variety of pharmaceutical formulations as an acidulant, flavor, and synergistic antioxidant and sequestering. At the laboratorial and industrial territories, due to safety precautions, phosphoric acid may be considered a valid acid alternative for stronger and risky acids such as sulfuric, hydrochloric, and nitric acids. Furthermore, phosphoric acid, among the mineral acids, is less corrosive for steel and all goods made therefrom. Taking into account all these favorable arguments, the applied research at our laboratory (LQBB) is focused, with success, in the utilization of much diluted and moderately thermopressurized phosphoric acid (*o*-PA) in the pretreatment of polysaccharides for many biotechnological, as oligosaccharides production, important prebiotics for the human gastrointestinal tract.

Keywords: phosphorus, phosphoric acid, oligosaccharides, prebiotics, polysaccharides depolymerization

1. Introduction

Henning Brand, a German merchant, when distilling urine discovered phosphorus and historically registered it, in 1669, through a communication to the famous chemist Gottfried

Wilhelm Leibniz. The addition of sand or coal to urine facilitated to turn phosphorus free. However, the first industrial sources for phosphorus production were bones previously converted into phosphate with nitric or sulfuric acid and then again treated with coal. This has been the contribution of Carl Wilhelm Scheele, in 1770. Much before, Arabian chemists had described a similar process also using urine. Etymologically, the word “phosphorus” was taken from Greek (“*phos*” + “*phoros*” = light + bearer) since it can be seen, in the darkness, when elemental phosphorus is exposed to the air [1, 2].

Overpassing several unsuccessful attempts, James Readman, at Edinburgh, Scotland, built an electric furnace where phosphate-rich materials mixed with carbon and silica generated free phosphorus. He transferred the patent rights to Albright & Wilson Co., from Oldbury, England, a company that started the industrial production of phosphorus and then exported the knowledge to France, Germany, Canada, and the United States [3].

Despite many technological improvements and huge scale up, the Readman method still holds and it consists of a strong reducing heating of fluorapatite with coke and sand at 1400–1500°C for the recovery of phosphorus vapors:



The energy demand of this electrothermic process is very high, around 14 MWh/ ton. Reason by which only a few countries with an abundant supply of hydroelectric power as is the case of Kazakhstan, China, and the United States use to adopt this technology despite the sources of fluorapatite mines being also widespread at other countries such as, Morocco, Tunisia and Russia. Usually, three vertical electrodes feed a modern furnace with the energy high input for the vaporization of P_4 from a mixture of coke, sand, and phosphate rock in a proportion of 16:30:100. A water spray followed by more cold water leads then to the condensation of P_4 , so a furnace unit with 12 m diameter and 8 m height can produce around 30,000 ton of white P/year. However, the whole process is not restricted to this short description. The presence of some silica, calcium, and iron in phosphate rock fluorapatite demands some parallel steps to conveniently and environmentally address these minor byproducts like ferrousphosphorus.

The other important P allomorph, red phosphorus, although easily ignited is not spontaneously flammable. Its production from white phosphorus is somewhat simpler: P_4 is maintained under a bed of water inside a closed steel reactor, heated at 288°C for a couple of days. The red product escapes to a lid pipe, followed by a condenser. Since the chemical nature is the same, the representative equation is: P_4 (white) \rightarrow P_4 (red).

A temperature elevation to 356°C heat allows the distillation of any residual white phosphorus whose boiling point (b.p.) is 289.5°C. Crystalline red phosphorus has a very high melting temperature (590°C).

Incorporation of phosphorus to ignite wooden splints impregnated with sulfur as precursors of the modern matches was carried by Robert Boyle, in 1680. The industrial practice led to a laboral facial deforming disease provoked in the workers by white phosphorus and then named *phossy jaw*.

2. Phosphorus: basic chemical and physical aspects

Phosphorus (P; in the Table of Elements: atomic number = 15; atomic weight = 30.973761998), and a very particular $3s^2 3p^3$ shell of electrons, may display as much as eight progressive states of oxidation: +5, +4, +3, +2, +1, -1, -2, -3, and -4 as a direct reflex of its electronic configuration [Ne] $3s^2 3p^3$. Phosphorus is so reactive that it allows several binary chemical combinations mostly neglected in the literature. Examples are its combination with halogens such as PBr_3 , PCl_5 , PI_3 , PF_5 , with nitrogen PN and P_3N_5 as well other divalent pairs (Ca_3P_2 , CuO_2 , and FeP/Fe_3P_2). Obviously, the expected combination with oxygen leads to several oxides (P_2O_3 , P_2O_4 , P_2O_5), in turn generating, upon hydration, the respective oxyacids. Given that sulfur may replace oxygen in some instances, phosphorus also combines with it to P_4S_3 and P_4S_{10} .

Phosphorus (P) is scored as the 12th more abundant element on Earth. In parts per million units, P is around 1050 ppm by weight or 730 ppm by moles of all mineral matter in the soil crust. About 190 different minerals possess P in their composition, the richest one being apatite, a combination with calcium, along with minor proportions of fluorine, chlorine, or hydroxide in a general formula $[Ca_{10}(PO_4)_6(F, Cl, \text{ or } OH)_2]$. The most important source for industrialization is phosphorite or phosphate rock mainly found in North Africa, Russia, and the United States what account for almost all of the 5×10^{10} tons available in the Earth's crust. P's single natural isotope is P^{31} although some artificial isotopes through nuclear reactions like P^{32} (the only one stable) are very useful for research purposes given its very short half-time life: only 14.3 days and then the radio mapping of several metabolic pathways in all living organisms as exemplified by DNA, RNA, glucose and fructose-P (the feed for the glycolytic pathway), and adenosine triphosphate (ATP), the energetic coin and P-lipids of any cell membrane. Furthermore, phosphate is the main electrolyte in the human plasma and is between 1.12 and 1.19 mM L^{-1} , and there are around 750 g of P in the human body. P is so reactive that it is not found in the nature as its single element. Rather, when reductively produced in the laboratory, its names are white and red phosphorus. White phosphorus when reacting with air oxygen generates a glow formerly known as *phosphorescence* and nowadays as *chemiluminescence*. Other allotropic forms of the element are "scarlet or violet" and "black" phosphorus, the latter a conductor of electricity. All these P allotropes are crystalline from cubic to orthorhombic types. These are very dense substances with an average $d = 1.828\text{--}2.69 \text{ g cm}^{-3}$. The tetrahedral white phosphorus given its flammability and pyrophoricity was used as one of the ingredients of "war weapons" that the USA used in Vietnam and other countries under military conflicts.

Phosphorus (P) as arsenic (As) as well as nitrogen (N), bismuth (Bi), and antimony (Sb) is said to be a pnictogen (five electrons in the outermost shell) and its 0.1% natural occurrence make it the most abundant pnictogen among the five in the terrestrial crust [4].

3. Phosphorus-derived oxoacids

The oxoacids derived from phosphorus display three particular atomic aspects: acidic protons and -P-P-links and sometimes, nonacidic hydrogen directly linked to P. The 10 most well-known occurrences or synthetic derivatives as oxyacids from P are:

(a) Valence +5 P (and acidic protons)

- H_3PO_4 orthophosphoric acid (3)
- $\text{H}_4\text{P}_2\text{O}_7$ pyrophosphoric acid (4)
- $\text{H}_5\text{P}_3\text{O}_{10}$ tripolyphosphoric acid (3)
- $\text{H}(\text{HPO}_3)_n\text{OH}$ polyphosphoric acid ($n + 2$)
- $(\text{HPO}_3)_n$ metaphosphoric acid (n)

b. Valence +4

- $\text{H}_4\text{P}_2\text{O}_6$ hypophosphoric acid (4)

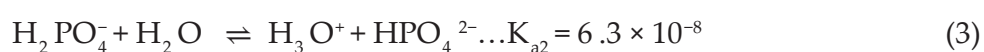
c. Valence +3

- H_3PO_3 (ortho)phosphorous acid (1)
- HPO_2 metaphosphorous acid (1)
- H_2HPO_3 phosphorous acid (1)

d. Valence +1

e. HH_2PO_2 hypophosphorous acid (1)

The most prevalent compounds of phosphorus are derivatives of phosphate (PO_4^{3-}), a tetrahedral anion. Being triprotic, phosphoric acid converts stepwise to three conjugate bases as a consequence of its progressive pK_{a1} , pK_{a2} , and $\text{pK}_{a3} = 2.15, 7.20$, and 12.35 :

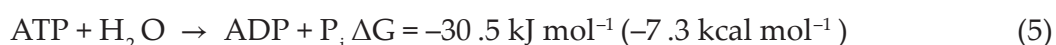


4. Phosphorus, phosphoric acid, and its esters: their outstanding importance in the biological life and environment

Although the most intensive if not relevant application of phosphorus is as phosphate-based fertilizers, one should recall that any living matter from a bacterium to the man is vitally dependent on phosphate since a lot of noble and essential biochemical molecules are built on phosphate: nucleic acids (DNA and RNA) as their nucleotide-based backbone is bridged by phosphodiester linkages; the life most important energetic coin, adenosine triphosphate (ATP); glucose- and fructose-P, the feeding fuels for the universal glycolytic, or

Embden-Meyrhoff-Parnas pathway which requires the previous activation by phosphorylation of the neutral hexose pair prior to their entry in EMP pathway; phospholipids in the assembly of cell membranes; and the serine-P differential aminoacid in casein, the perfect food for newborn. As a critical example, cholesterol biosynthesis' (and by extension several hormones derived therefrom) first steps are the sequential building of mevalonate-mono- and di-phosphate. Furthermore, phosphorylation (as well dephosphorylation) of key enzymes through the action of specific, also enzymatic catalysts (kinases), controls the "on-off" of whole metabolic pathways.

Furthermore, most of the metabolic pathways of the human, animal, and microorganism cells and tissues ask for the direct or indirect energy content of ATP (or other coenzyme analogs as NADP—nicotinamide adenine dinucleotide phosphate) to drive particular reaction steps. The energetic content of this coenzyme is elevated:



Human body, on average, contains 250 g of ATP given the permanent coenzyme recycling. Moreover, animal bones and teeth are mainly composed (70%) of a form of hydroxyapatite— $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ but with a Ca:P ratio between 1.37:1.87 instead of 5:3 as seen in the earth mineral. In a normal adult male human body (80kg), around 12kg are bones (dry weight basis). The proteins of these particular anatomic body pieces experience stiffening thanks to the complexation with calcium phosphate.

The whole metabolism of phosphate in any living organism involves the catalysis carried out by many enzymes. They are designed as kinases (building the phosphoric esters), pyrophosphorylases (synthesizing import sugar donors for polymers), isomerases (changing, for instance, a sugar phosphate hexose, G-6-P, to the similar ketose-6-P), and phosphatases (hydrolyzing phosphate esters). From both the anabolic and catabolic standpoints, the pool of free phosphate, shortly P_i , corresponds to the bioavailability of the cosubstrate for the energetic premobilization of glucose units present in reserve polysaccharides. In this way, glycogen from animals, yeasts, and molds, and starch from plants and algae are sequentially catabolized to glucose-1-P.

Among pathologies of medical interest, hypophosphatemia is a condition of low levels of soluble phosphate levels in the blood serum, and therefore inside cells. Symptoms of hypophosphatemia include neurological dysfunction and disruption of muscle and blood cells due to lack of ATP. Low-phosphate syndromes are caused by malnutrition, by failure to absorb phosphate, and by metabolic syndromes that draw phosphate from the blood (such as refeeding after malnutrition) or pass too much of it into the urine. Conversely, hyperphosphatemia is characterized by too much phosphate leading to diarrhea and calcification (hardening) of organs and soft tissue, and also disturbing the normal body's ability to absorb and utilize iron, calcium, magnesium, and zinc.

Around a half century ago, DIPFP—diisopropylfluorophosphate ($\text{C}_6\text{H}_{14}\text{FO}_3\text{P}$.) was in medical use as eye drops do alleviate symptoms of glaucoma-associated ocular hypertension but the consequent eyes opacity precluded this use.

At the molecular level, DIPFP acts as a potent toxin since it combines with serine at the acetylcholinesterase active site thus affecting the normal metabolic interconversion of the neurotransmitter acetylcholine. Furthermore, DIPFP also inactivates some proteases. Its LD_{50} in rat is as low as 6 mg kg^{-1} when administrated orally. This explains its intended use as military weapon by ancient British researchers [5, 6].

Notwithstanding its nomenclature scope, an IUPAC publication gives an excellent sight of the reach of phosphorus/phosphate in natural compounds from any kingdom [7].

5. Natural occurrences of phosphorus and its association with other elements

As a direct consequence from its natural chemical properties and reactivity, the natural occurrence of phosphorus—rocks and minerals—corresponds to the more oxidized forms of the element, the inorganic phosphates (PO_4^{3-} or shortly, Pi), and main fertilizers for plants. Apatite (a calcium phosphate) is its main mineral and does occur with another nature wealth—petrol—this mineral is mainly present at Arabian countries. Florida, USA, Northern Africa, China, and Russia are also important sources of P rocks. The estimate of United States Geological Survey (USGS)—a geological service is around 71 billion tons but at the actual rate of mining and consumption as fertilizer and production of industrial phosphoric acid (1.5 billion ton/year), phosphate minerals may turn exhausted in 4 or 5 decades from 2012. Interestingly, guano (marine seagull and other related birds feces and animal bone ash) have been also explored for phosphorus sources. In fact, the modern technology now thinks about man and animal urine management for the recovery of both phosphate and nitrogen as fertilizers, thus minor-ing the progressive eutrophication of rivers and lakes. Most of the intensive agriculture relies in the adequate phosphate fertilizers supply to soil. Superphosphate is a special formula for such a purpose and it corresponds to a blend of calcium dihydrogen phosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2$] and calcium sulfate dehydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). P^{31} is a natural stable isotopic occurrence of phosphorus that is radioactive and is very useful for the investigation of phosphorylated compounds through the spectroscopic technique known as ^{31}P -NMR (nuclear magnetic resonance). Conversely, radiochemistry takes advantages of radioactive occurrences of phosphorus as β -emitters. For instance, P^{32} and P^{33} with half-lives of 14.3 and 25.4 days and energies of 1.71 and 0.25 MeV is useful for research on DNA and RNA probes and their respective Northern and Southern blots as well as DNA sequencing.

6. Production and direct uses of phosphorus

Among other allotropic forms of phosphorus, only two forms have commercial significance—white and red phosphorus—the former even more important given it accounts for around

99% of the world demand. Some food and pharmaceutical processing as well for etching semiconductors require high purity phosphoric acid (H_3PO_4 ; more strictly designed as *ortho*-phosphoric acid, usually at 85% w/w) also known as “thermal phosphoric acid” which is made by burning phosphorus in moist air. Conversely, phosphoric acid destined to the huger demand of fertilizer production does not require high purity. Hence, it is directly obtained from rock phosphate ores without the intermediary step of elemental P.

Historically (latter half of nineteenth century), the alternative source of phosphate was guano (the excrement of seabirds and bats accumulating in isolated islands as in Chile), a major form of P-based fertilizers lime superphosphate, a mixture of two salts, calcium dihydrogen phosphate $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and calcium sulfate di-hydrate $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, produced by the reaction of sulfuric acid and water with calcium phosphate.

The profile of the participation of phosphorus derivatives/products in the industrial activity is shown in **Figure 1**.

Some forms of phosphorus, although low in demand, have very specific applications. For instance, red phosphorus is employed in pyrotechnics and matches and even as fire retardant in plastics like polyamides as it quickly consumes oxygen, the natural oxidizer in burning. World annual production of elemental phosphorus accounted (in 2013) for 850,000 tons [8].

White phosphorus is used as a deoxidizing agent in the preparation of steel and phosphor bronze. Phosphoric acid is primarily used in the production of phosphate compounds. It is also used in pickling metals, in sugar refining, and in soft drinks. Phosphorus forms a number

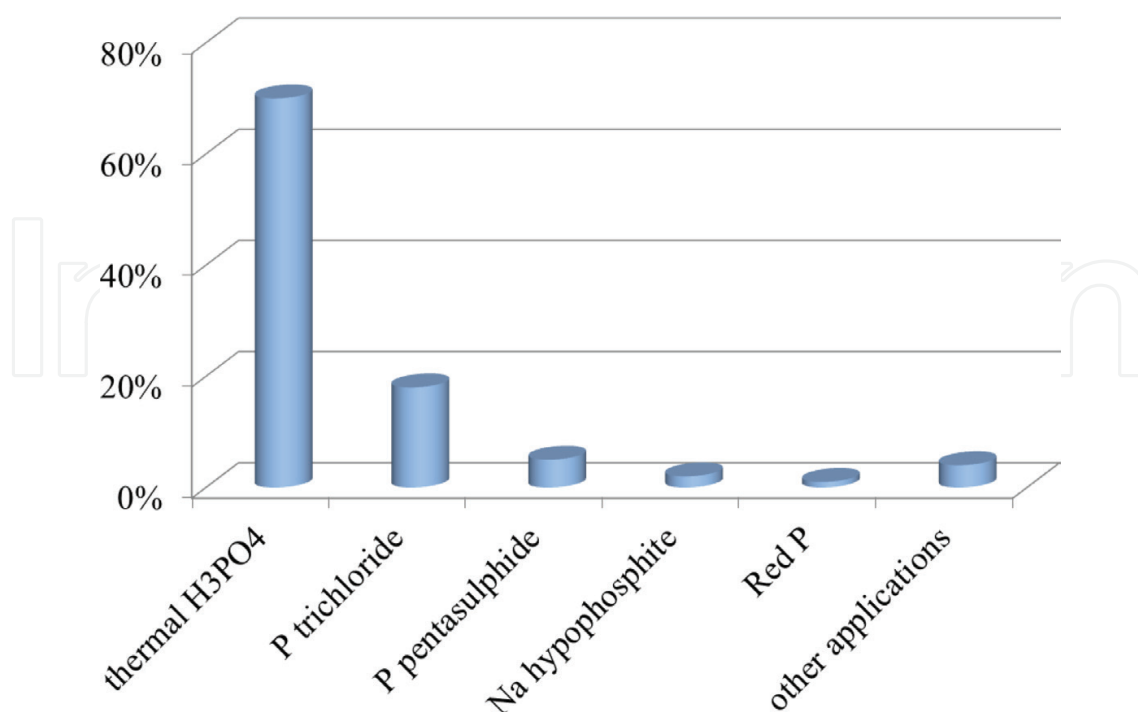


Figure 1. Industrial uses of phosphorus and P-derivatives.

of compounds with halogens, e.g., the trichloride, PCl_3 , and the pentachloride, PCl_5 , both used as reagents. It also forms an oxychloride, POCl_3 . It reacts with sulfur to form a pentasulfide, P_2S_5 , and a thiochloride, PSCl_3 , used in insecticides and oil additives. Phosphine, PH_3 , is a very poisonous gas.

The lighting effects and colors of fireworks came from their chemical components under burning. Aluminum generates white and silver flames; antimony intensifies bright color, while calcium and barium lead to orange and green colors. Magnesium, copper, lithium, and sodium create bright white, blue, red, and yellow colors, respectively. Iron, depending of the heating intensity, converts into brown to red brown sparks. Zinc is the component for generating smoke. Powdered carbon, along with some sulfur, is the fuel for fireworks and the energy of its burning acts as propellant for all other colors and forms generating components. Herein, phosphorus may turn a key element giving to the night darkness a special glowing effect and also helping the propellancy.

In the dentistry field, trimetaphosphate finds use to reduce the fluoride content of dentifrices without losing its anticariogenic properties. Mouth environmental pH, if reduced, increases the deposition of both fluoride and phosphate on teeth enamel. A reduced mineral loss from teeth results from the incorporation of sodium metaphosphate in dentifrices. The role of several phosphate salts alone or in combination with fluoride has been extensively reviewed and the benefits stand very well established [9–12].

Phosphorus-derived compounds have a wider range of industrial applications. One example is triaryl phosphates in the improved properties they confer to hydraulic fluids concerning lubrication and fire retardation [13].

High-pressure and antiwear properties in EP additives (extreme pressure) in greases, gear oils, and motor oils are benefits arising from oil-soluble organophosphates, with or without zinc, since they provide corrosion protection especially in presence of chlorinated hydrocarbons. For instance, the coordination compounds zinc dialkyldithiophosphates (ZDDP), also antioxidants, start decomposing at 130–170°C, while the activation temperature of tricresyl phosphate (TCP) typically exceeds 200°C. Their reaction products form a chemically bonded lubricating film on the surfaces. TCP has other uses as well: plasticizer in nitrocellulose, lacqueurs, and varnishes and flame retardant in plastics and rubbers [14].

Due to huge material losses (and human, as well) in case of domestic, industrial, and field fires, phosphorus-based flame retardants are being actively developed to provide more appropriate substitutes for the classic brominated flame retardants. So, aryl phosphate dimers and oligomers turned appropriate for plastic flames such as polycarbonate- and polyphenylene-styrene blends. Polyurethane flames are better controlled with an oligomeric aliphatic phosphate. Moreover, polymeric phenylene methylphosphonate and cyclic phosphinates fit better from fires coming from epoxy resin-impregnated boards [15].

Concerning the extraction/purification technologies for crude phosphoric acid, many of them are available for the solvent-based approaches and they are known as the name of their proponents/inventors/factories: Albright & Wilson; Budenheim, FMC, IMI, Prayon, Rhone-Poulenc, Bateman-Wengfu/Prado-AFB Turkey [16].

6.1. Applications and uses of aluminum phosphate A: $\text{Al}(\text{H}_2\text{PO}_4)_3$

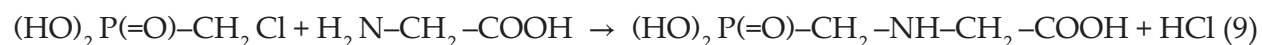
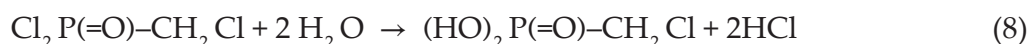
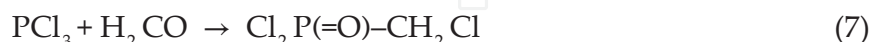
Aluminum is one of the most common metals found in nature in the form of the minerals based on aluminum phosphate (e.g., angelate, coeruleolactate, evansite, lucinite, sterretite, variscite, wavellite). Aluminum phosphate is formed when the phosphoric acid reacts with alumina present in the catalyst. Its industrial applications are varied: as cement in admixture with calcium sulfate and sodium silicate, as a flux for ceramics, in dental cements, waterproofing concrete, as a flame retardant, as a catalyst in organic synthesis, and for special glasses. Aluminum phosphate is also employed as emollient in cosmetics, relieves stomach ailments in the case of heartburn or peptic ulcer pain, and to control excess sweating. Environmentally, the effects of aluminum phosphate are not classified as an acute or chronic toxin to aquatic life or the environment. As an inorganic substance, aluminum phosphate is not biodegradable. Neither bioaccumulation nor biomagnification are considered to be environmental issues [17].

7. The massive agricultural use of a special form of phosphorus: phosphonate-based herbicides

Although a subject that evokes permanent controversy, one of the most intensive industrial applications of phosphoric acid is the chemical synthesis of glyphosate or N-phosphonomethylglycine, invented, produced, and distributed throughout the world by Monsanto with the commercial name "RoundUp®" [18].

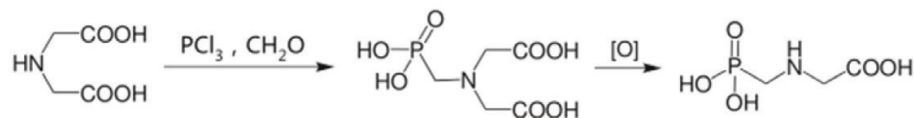
It is an interesting and selective herbicide against large-leaf and grassy weeds. In tillages based in the transgenic soya "RoundUp Ready," which are not sensitive to glyphosate, are thus easily turned free from its common weeds thanks to the nebulization with glyphosate. From the chemical standpoint, in glyphosate it occurs as one of the strongest chemical links—phosphonate—a direct linkage between C and P. Since glycine composes most of the proteins and enzymes and has other important anabolic roles in any live organisms (one C donor, precursor of glutathione, creatine, hemoglobin, and purine nucleosides), it is comprehensive how its chemical modification toward a phosphonate derivative is so efficient herbicide [19].

Historically, and at the lab scale, glyphosate was synthesized as follows:



Nowadays, glyphosate's industrial production is attained by one of the following pathways, shown in **Figure 2**.

A. Iminodiacetic acid and phosphorous/hydrochloric acids:



B. Dimethylphosphite + bis-hydroxymethylglycine

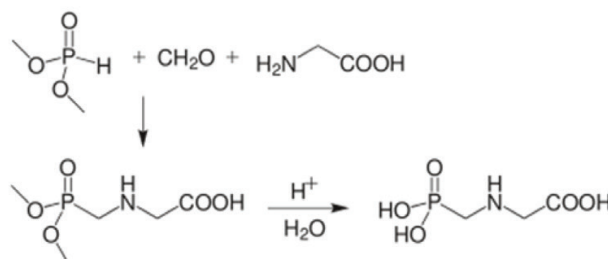


Figure 2. Modern routes for industrial production of glyphosate (N-phosphono-methyl-glycine).

Some of the aspects previously mentioned as controversy can be drawn from three recent review. A 2013 review found that neither glyphosate nor typical glyphosate-based formulations (GBFs) pose a genotoxicity risk in humans under normal conditions of human or environmental exposures. A 2014 review article reported a significant association between B-cell lymphoma and glyphosate occupational exposure. In March 2015, the World Health Organization's International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological studies, animal studies, and *in vitro* studies [20–22].

There is a plenty of other P-containing herbicides and insecticides—generally said, pesticides sharing the double Janus-faces. On one side, ensuring better crops and agriculture productivity; on the other, scavenging the health of people managing with or consuming contaminated crops, animal food, and water. Other organophosphates (OPI) maintain as the most widely used group of insecticides in the world, despite their toxicity not restricted only to target harmful insects in the agribusiness. Human and animal are the subsequent victims due to the accumulation in soil and waters and their further feeding of contaminated vegetables, fruits, milk, food products, and other living organisms, and it is not an important concern as the profits are coming from the agricultural activity. OPI's main target is the inhibition of the enzyme acetylcholinesterase (ACE), a key enzyme in the ana- and catabolism of the most important neurotransmitter, acetylcholine (Ach), in the brain/nervous system of animals. Following the accumulation of Ach, an overactivation of cholinergic receptors does occur at the neuromuscular junctions as well in autonomic and central nervous system [23].

The general chemical structure of OPI is as follows (**Figure 3**):

Malathion and Dichlorvos, encompassing chemical combination with sulfur and chlorine, respectively, and two examples of most utilized organophosphate pesticides. About a decade ago, the agricultural market used to offer >900 pesticides and >600 active pesticide ingredients.

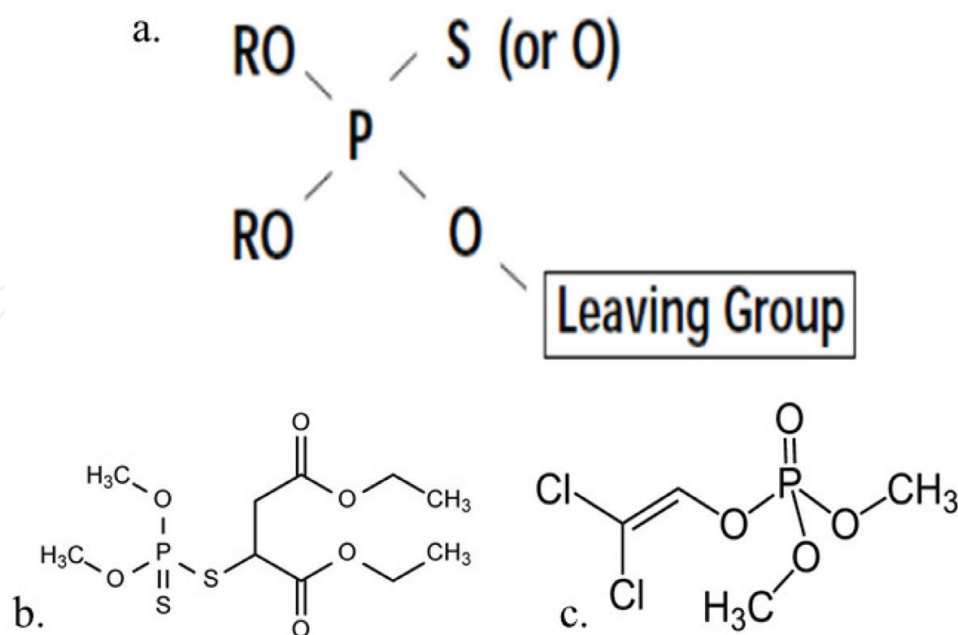


Figure 3. Chemical structure for organophosphorus insecticides (OPI).

Acute toxicity of OPI varies widely. Following is the example of increasing intoxication, and expressed as LD_{50} ranges, chlorfenvinphos (1–30) > dichlorvos (30–50) > malathion (60–1300 mg/kg body weight) [24].

Another particular utilization of phosphorus (white P) is for the production of military weapons such as grenades, mortar shells, and artillery shells for related purposes such as smoke screens for troops movement, marking targets, bullets path tracing, and, worse, as incendiary ammunitions. Viet Cong Tay Ninh province in 1967 and Fallujah at Iraq (2007) experienced the destroying power of these U.S. ancillary weapons. It is worth mentioning that mixtures of phosphoric acid with nitromethane are explosive [25].

8. Environmental utilization of phosphorylated matrices

Phosphorylation of several matrices for multiple purposes as environmental remediation of cationic residual compounds (e.g., cationic dyes and antibiotics) may proceed in the cases of acid-resistant materials (e.g., activated carbon) through the treatment of previously oxidized matrix with a mixture of sulfuric and phosphoric acids or even with the latter alone, provided it is converted in polyphosphoric acid through the chemical removal of the usual moisture of H_3PO_4 85% with the stoichiometric amount of the P anhydride, P_2O_5 . For more delicate matrices (e.g., cellulose), a gentle alternative is the utilization, for the same purpose, of cTP—cyclic-Tri-Phosphate, which is easily prepared from potassium dihydrogen phosphate in a muffle at 450°C for 6 h. The next phosphorylating step is carried out at room temperature for 1 h at a moderate alkaline solution with pH 9–10 leading so to the desired products such as phosphorylated activated carbon or cellulose (AC- Pi^{2-} , cellulose- Pi^{2-}), and so on. Polyphosphoric

acid is useful for as much as eight different industrial applications: cyclization reactions, rearrangements, dehydration, hydrolysis, polymerization, solid phosphoric acid catalyst, polyamide yarns processing, and polymerization [16].

Particular applications of phosphoric acid in activation in carbonaceous matrices may provide interesting solutions for some contaminations, as is the case of water by the ammonia from human and animal urine. So, activated charcoal impregnated with H_3PO_4 is efficient to remove and modify ammonia gas and hydroxide according to Ref. [26]:



In fact, besides sulfuric acid, phosphoric acid is the common chemical tool to convert impure mined charcoal into activated charcoal [27, 28]. In an opposite sense, excess of phosphate salts discharged in aquatic streams and lakes leads to eutrophication and sometimes to microalgal blooms, as a result of the rupture of normal and harmonic life cycles of several microorganisms [29].

9. Biochemical and pharmaceutical uses of phosphoric products

Concerning the utility of phosphate salts in more sophisticated biochemical applications, once the ionic liquid choline dihydrogen phosphate was demonstrated as an improver of thermostability and shelf-life of several proteins and hence its usefulness as an excipient for the formulation of protein-based pharmaceuticals, the toxicity of several analogs were assayed using a murine macrophage cell line. EC_{50} values were found to be closely related to safe salts (e.g., choline chloride), which indicated feasibility for the intended therapeutic proposal provided the anionic counterpart is not too much larger [30].

Phosphoric acid is also widely used as an acidifying agent in a variety of pharmaceutical formulations. It is used in pharmaceutical products as part of a buffer system when combined with a phosphate salt such as sodium phosphate, monobasic, or dibasic. It is also widely used in food preparations as an acidulant, flavor, and synergistic antioxidant (0.001–0.005%) and sequestering.

Therapeutically, dilute phosphoric acid has been used well diluted in preparations used in the treatment of nausea and sickness. Phosphoric acid 35% gel has also been used to etch tooth enamel and to enhance delivery of drugs through the nail. Nanosized hydroxyapatite powder was made by combining phosphoric acid with eggshells.

The lowest lethal oral dose of concentrated phosphoric acid in humans is reported to be 1286 mL kg^{-1} . LD_{50} (rabbit, skin): 2.74 g kg^{-1} and LD_{50} (rat, oral): 1.53 g kg^{-1} .

In the UK, a 1 in 330 aqueous solution of phosphoric acid is approved as a disinfectant for foot-and-mouth disease. A specification for phosphoric acid is contained in the food chemicals codex (FCC). The EINECS number for phosphoric acid is 231-633-2. The PubChem Compound ID (CID) for phosphoric acid is 1004 [31].

Tableting is one of the largest applications of calcium phosphates in the pharmaceutical field. Due to the different surface areas, calcium salts of phosphoric acid strongly differ concerning their physical properties. For instance, at 93% relative humidity, most of hydroxyapatites can absorb 15% of moisture while the nonhygroscopic basic calcium phosphate dehydrate absorbs <1% [32].

A net mineral loss in the teeth led to porosity, white-spot lesions, caries lesions, and cavitation. The modern approach to circumvent these inconveniences is the remineralization through the application of nanoscale-structured calcium phosphate prepared via the spray-drying technology and the results are encouraging. A typical formulation is calcium and phosphate concentrations around 8 and 5.333 mM L⁻¹ after the appropriated blending of anhydrous dicalcium phosphate and calcium carbonate in diluted acetic acid [33].

Given its three strategic dissociation constants ($pK_{a1} = 2.15$, $pK_{a2} = 6.82$, and $pK_{a3} = 12.38$), phosphoric acid ensures one of the widest ranges of buffering capacity for laboratory buffers and biological media through the appropriated mixture of its Na or K salts. Examples are McIlvaine (citric acid and Na₂HPO₄ from pH 2.6 till 7.6), Gomory/Sorensen (Na₂HPO₄ and NaH₂PO₄ from pH 5.8 till 8.0), Clark and Lubs (KH₂PO₄ and NaOH from pH 5.80 till 8.0), Bates and Bower (Na₂HPO₄ and NaOH from pH 11.0 till 11.90) [34].

9.1. Bisphosphonates and its odontological use

The sodium salt of alendronic acid is nowadays a bisphosphonate commercial product utilized to correct several bone diseases such as osteoporogenesis and osteogenesis imperfecta. Its beneficial effect arises from the inhibition of osteoclast-mediated bone resorption without effect on bone remineralization as is the case of pyrophosphate. The only aid is to avoid a parallel hypocalcemia [35–37].

9.2. Uses of phosphorus nonoxygenated compounds

The main utilization of phosphoric acid is the manufacture of fertilizers. Mono- and di-acidic calcium phosphates are employed as additives in the fabrication of toothpaste, animal food, baking powder, and fertilizers. Na₅P₃O₁₀ is preferentially used in detergents. Conversely, phosphorus trichloride (PCl₃) and its oxygenated derivative POCl₃ are used in the manufacture of pesticides and plasticizers. For the former proposal, phosphorus sulfide (P₄S₁₀) is used as well.

10. Phosphoric acid and phosphate salts in foods and beverages

Phosphoric acid (as a mild acidulant) and phosphate salts are also used to control pH of processed foods. They are also used in medicines for constipation and to prepare the bowel for medical procedures. Two of the most remarkable commercial products where flavor intensity (e.g., arising from sweeteners) is valorized by phosphoric acid or its mix with phosphate salts are the colas and fermented milk, as shown in **Figure 4**.



Figure 4. Illustrations for beverages (colas) and fermented milk (yogurt) and their phosphoric acid or P-salts or citric acid ingredients.

In the bakery business, sodium phosphates are often used as emulsifiers, thickening agents, and leavening agents for baked goods. They are also used to control pH of processed food procedures.

Syrup phosphoric acid is very useful to stop or correct oxidized metallic pieces made of iron or steel. Acid application leads to the formation of a barrier pellicle of iron phosphate and hence its antirust action.

Trisodium phosphate is used as a cleaner, water softener, and scale/corrosion inhibitor. Bone ash (calcium phosphate) is used to make chinaware and to make monocalcium phosphate for baking powder.

Sodium tripolyphosphate, shown in **Figure 5**, is used in laundry detergents in some countries, but banned in other countries. It is useful for softening water to enhance the performance of the detergents and to prevent pipe/boiler tube corrosion.

The use of phosphate salts which nowadays turned completely obsolete is in the fabrication of sodium lamps since their replacement by fluorescent lamps and light-emitting diodes (LED) is now consolidated for the sake of energy expenditure cutting.

11. Utilization of diluted, heated, or thermopresurized (ortho) phosphoric acid (*o*-PA) as a mild catalyst for an advantageous phytobiomass polysaccharide partial or total depolymerization

At the laboratorial and industrial territories, due to safety precautions, phosphoric acid may be considered a valid acid alternative for stronger and risky acids such as sulfuric, hydrochloric, and nitric acids, since its more common commercial form, 85% (w/w) H_3PO_4 is really safer.

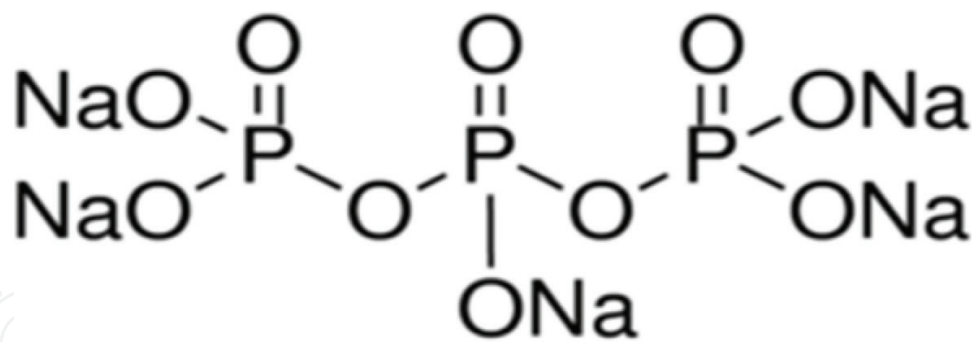


Figure 5. Sodium tripolyphosphate formula.

HCl (37% w/w) and HNO₃ (65% w/w) both evolve as very harmful vapors to the eyes, skin, and lungs while H₂SO₄ (96–98% w/w), given its deep avidity for water, leads to quick carbonization of organic matter and severe wounds to the body, in case of accidents. Another very common utilization of phosphoric acid is in the formulation of buffers (mixes of its Na⁺ or K⁺ mono- and di-acid salts) as it is equally true for its buffering occurrence in the blood stream of any animal species. Furthermore, phosphoric acid, among the mineral acids, is less corrosive for steel and all goods made therefrom.

Taking into account all these favorable arguments, the applied research at our laboratory (LQBB—Biomasses Chemo/Biotechnological Laboratory at UTFPR—Federal Technological University of Paraná, Curitiba-PR, Brazil) is focused, by decades, in the utilization of very diluted and moderately thermopressurized phosphoric acid (*o*-PA) in the pretreatment of polysaccharides for many biotechnological purposes. Sequentially, the selected substrates were and are hemicelluloses (hetero-xylans from cane bagasse), inulin (a labile polyfructose from dahlia tubercles), cassava starch, and, more recently, hetero-mannans from conifer woods sawdust and commercial seed gums. Homo- and heteropolysaccharides present in phytobiomasses display different lability when facing hot concentrated or diluted mineral acids as consequence of the carbohydrate units (hexoses, pentoses, deoxysugars, acid, and animated hexoses) and even more from the type of glycosidic links between monosaccharide units and their anomericity. For instance, the β-1,4 links of the pyranosidic links of glucose units in cellulose and N-Acetil-glucosamine, respectively, render both very resistant to the hydrolytic action of acids. Usually, a prestep of the polysaccharidic substrate in cold concentrated acid (e.g., 60% w/w), followed by acid dilution (2–4 M) and heating allows the best free monosaccharide recovery. Obviously, the mildest alternative is the use of specific enzymes, namely, cellulases and chitinases, respectively.

In the following descriptions, the authors' experience on the monomerization of polysaccharides contemplating the extremes of natural examples of acid-lability: inulin (the extremaly labile poly β-2,6-fructofuranosyl); starch (a moderately labile poly-α-1,4-gucopyrasyl with a few α-1,6-branches); heteroxylan (also moderately resistant β-1,4-poly-D-xylopyranosyl backbone) with a few of single α-1,3-L-arabinofuranosyl extremely labile lateral units, the same for a few very resistant (4-O-methyl)-D-glucuronopyrasyl units, thus generating aldo-biuronic acid); and cellulose (the outstanding resistant and linear polymer of β-1,4-linked

glucopyranosyl units) is shown. For any of these particular cases, the diluted *o*-PA acid solutions are better expressed in terms of their effective pH before hydrolysis (after a certain time of equilibration with phytobiomass components) instead of the usual concentration parameters as % (v/w, w/w or mM L⁻¹). As a clever laboratory practice, the explored kinetical and severity parameters may optimize varying phytobiomass mass: diluted *o*-PA (e.g., till 40% in case of inulin and starch; only 10% in case of native ligno(hemi)cellulosics), time of residence in the reactor and more importantly the above-mentioned effective hydrolysis pH (e.g., 3.5–1.5) combined with the thermopressurization (peak temperature and corresponding pressure expressed in atmospheres) vary variable in the range from 60 to 200°C.

11.1. Inulin

Inulin, usually from *Dahlia* tubercles (although also present in chicory, yacon potatoes, and Jerusalem-artichoke roots), with an average degree of polymerization (DP) = 33, is not soluble in cold water as opposed to its complete solubilization in warm water. For pure inulin preparation, tubers from *Dahlia* spp. (e.g., *pinnata*) are washed to remove adherent soil particles, peeled, and quickly submerged in boiling water previously buffered with 25 mM pH 7.0 sodium phosphate to avoid any damage in the polyfructose native structure and the browning from phenoloxidases action. The hot suspension is comminuted in a Waring blender and the suspension is filtered through a 4× layers of cheese cloth. Upon refrigeration at 4°C overnight, inulin settles and may be recovered by centrifugation and dehydration with absolute ethanol and then acetone. The pure polysaccharide should be a white powder or at most, light cream. If still with a more brown hue, its warm solution may be filtered by a bed of DEAE⁺-cellulose for the removal of any residual colored matter.

Inulin is unique among all natural polysaccharides since it lacks a reducing end given its biosynthesis starts from a single sucrose unit (**Figure 6**). In fact, the presence of this minute amount of free glucose in any inulin hydrolysate is the trustable indicative one is managing the intact polysaccharide structure, as depicted. This may be easily confirmed by ¹³C-NMR since the smaller signals of the single glucopyranose units can be surely detected among the major signals arising from the fructofuranose main units as shown in **Figure 7**.

Inulin partial acid or enzymatic hydrolysis may be quickly monitored even for several samples by thin layer chromatography (TLC) as shown in **Figure 8**. A sharper profile of Fructooligosaccharides (FOS) generated upon *o*-PA or citric acids moderated and partial hydrolyses at pH 2.5 and 85°C for 5 or 15 min is gained with a high performance liquid chromatography (HPLC) shown in **Figure 9**.

Our factorial design prospection indicated the ideal conditions of inulin with *o*-PA regarding the obtention of the higher ratio FOS [FOS: free fructose + hydroxymethylfurfural (HMF)]: pH 2.5–2.0, temperature from 85 to 95°C (preferably the former), and shorter residence times of reaction from 15 to 25 min. Interestingly, an alternative acid catalyst, citric acid, could afford similar hydrolytic results, provided small enhancements of the hydrolysis severity parameters were applied. It will be noted that for food industrial uses, for instance, in gasified beverages or cokes and yogurts, neither *o*-PA nor citric acid need to be removed from the sugar

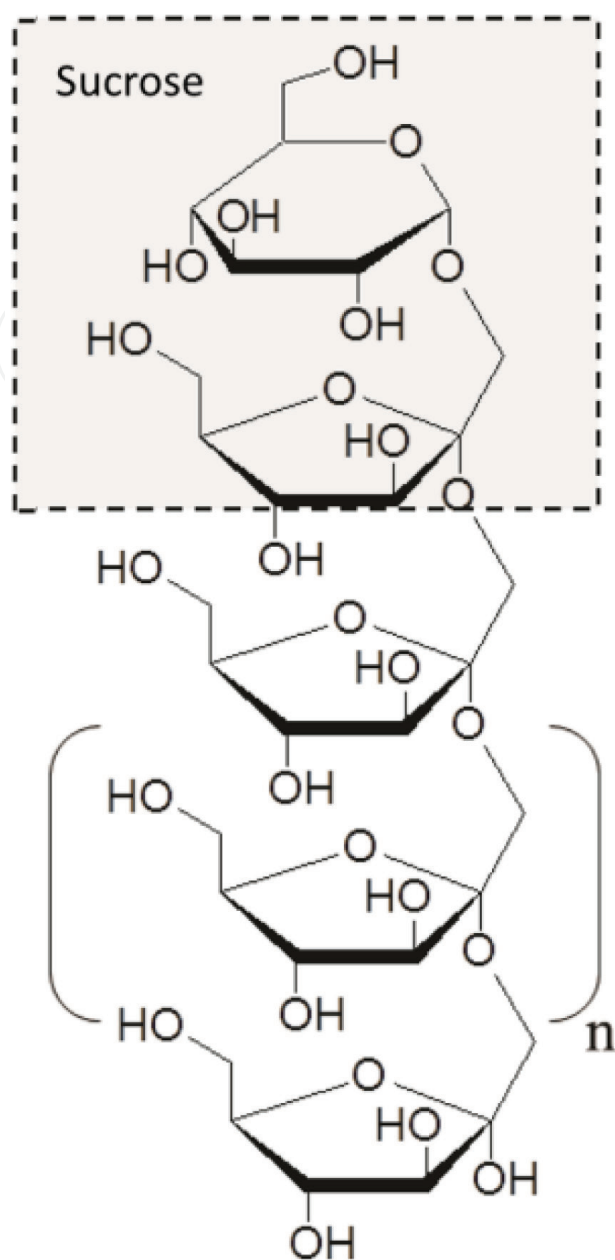


Figure 6. Simplified chemical structure of inulin, a β -linked polyfructofuranose.

partial hydrolysates, provided they are conveniently and partially neutralized to slightly less acid conditions (e.g., pH 3.5–4) with any desired bases (ammonia, lime, magnesium oxide, or even soda or potash). FOS, as extensively reported in the literature, are the most well-characterized nutraceuticals and particularly useful, when included in the human diet, blocking the appearance of colon tumors [38, 39]. **Figure 10** is a simplified summary of the factorial design leading to the best hydrolytic condition for the production of FOS from inulin. Herein, FOS are adopted as a family of the simpler fructobiose till larger oligosaccharides, DP = 17 or 18 and so on, and not simply the usual family of smaller FOS like kestose and neokestose mentioned in the literature and enzymatically obtained from transfructosylation of sucrose in a more expensive technology [40].

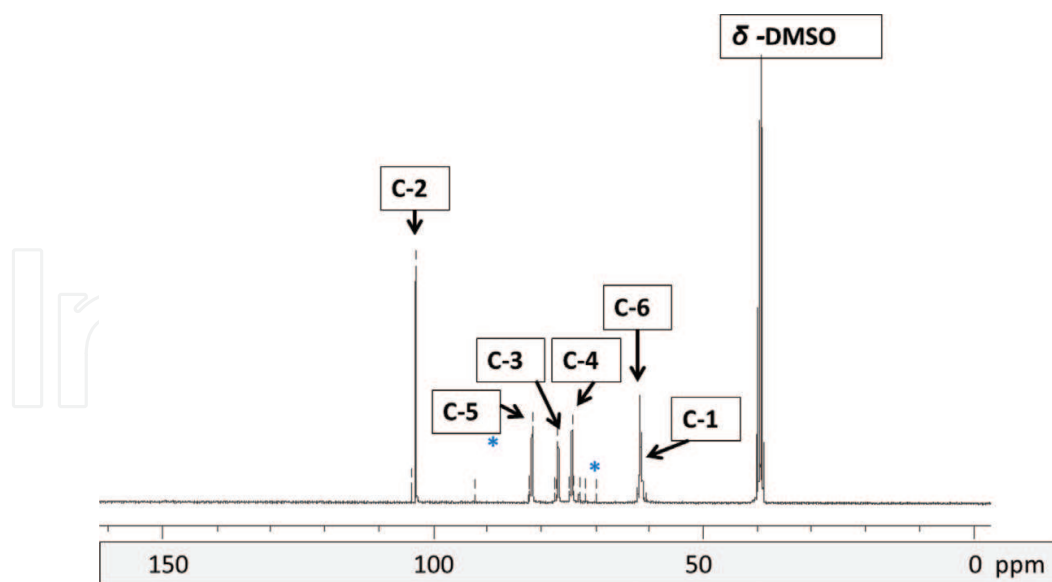


Figure 7. ^{13}C nuclear magnetic resonance spectrum of the purified inulin from *Dahlia* roots. Note: (*) minor signals arising from the single glucopyranose unit. Warm dimethyl sulfoxide (DMSO) as inulin solvent and ppm marker.

The phosphoric hydrolysate from inulin, optimized for FOS production, can be converted into short chain fatty acids (SCFAs), the real derivatives from FOS for the prevention of colonic tumors [41, 42].

11.2. Starch

Starch, the main reserve polysaccharide of many plants, fruits, and some algae, is a combination of linear amylose (exclusively α -1,4-linked glucopyranosyl linked) with α -1,6-branched amylopectin. Both of these glycosidic linkage types are moderately resistant to acid hydrolysis. We have prepared cassava starch purified granules by washing freshly collected roots, peeling, and comminuting them in a Waring blender and filtering the thick cold suspension through a 4 \times cheese cloth. After resting overnight, the starch grains settled and were again washed with distilled water for further lyophilization. Heavy starch paste was then pretreated with thermopressurized diluted phosphoric under selected conditions. The routine hydrolysis conditions varied from 2.8 to 6.8 atm, and the residence time at the peak temperature was from 5 to 10 min.

We have found that for cassava (manioc; *Cassava esculenta*) starch complete monomerization, the ideal conditions of thermopressurized hydrolysis, was polysaccharide concentration till 40% (w/v, *o*-PA effective pH = 1.75, and thermopressurization a 159°C/5 atm for a couple of minutes). HMF generation from glucose being released as free monosaccharide though its triple dehydration is lower when using *o*-PA than those generated at the same pH and severity hydrolysis parameters either using hydrochloric or sulfuric acids. This is graphically shown in the following **Figure 8**.

In the industrial jargon, the brown and bitter byproduct obtained when using these stronger acids is designed as “mud” and its elimination requires the use of expensive resins. Obviously,

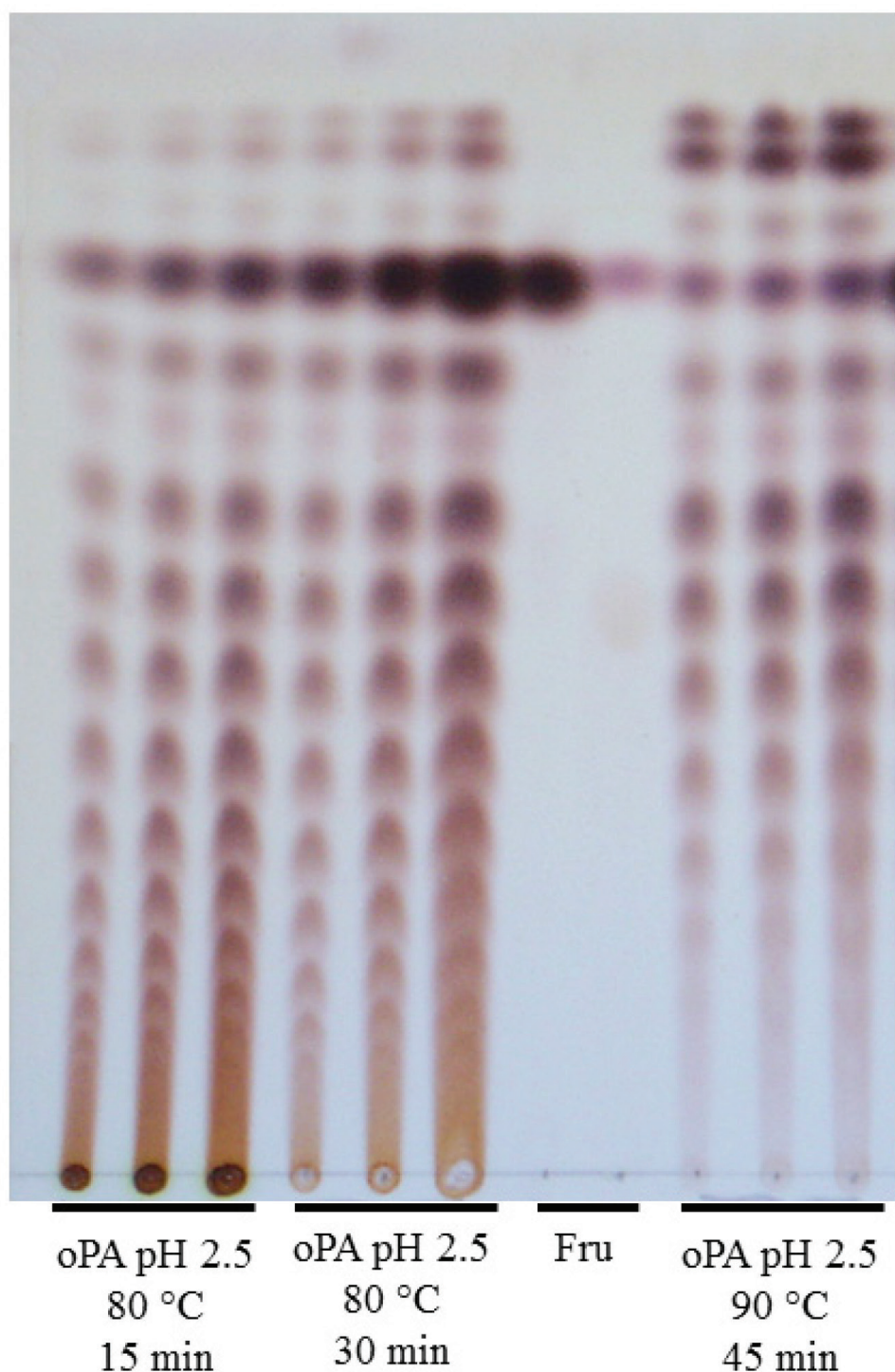


Figure 8. Thin-layer chromatographic analysis of the reaction products in the hydrolysis of inulin with phosphoric acid (*o*-PA) (pH 2.5) for 15, 30, and 45 minutes at 80°C. DP, degrees of depolymerization; FOS, fructo-oligosaccharides; Fru, fructose. Note: Silica gel 60 chromatoplates (Merck); isopropanol:ethyl acetate:water (5:2:1) was used as the mobile phase in three successive runs of 1/3, 2/3, and 3/3 of the front line, and hot 0.5 g% orcinol in sulfuric acid (10%) was used as the chromogenic agent).

o-PA technology allows modulation of the severity parameters to drive hydrolysis toward maltosaccharides instead of free glucose. It is worth to say that in comparison with inulin hydrolysis options (actually the industrial production of inulinases or fructofuranosidases

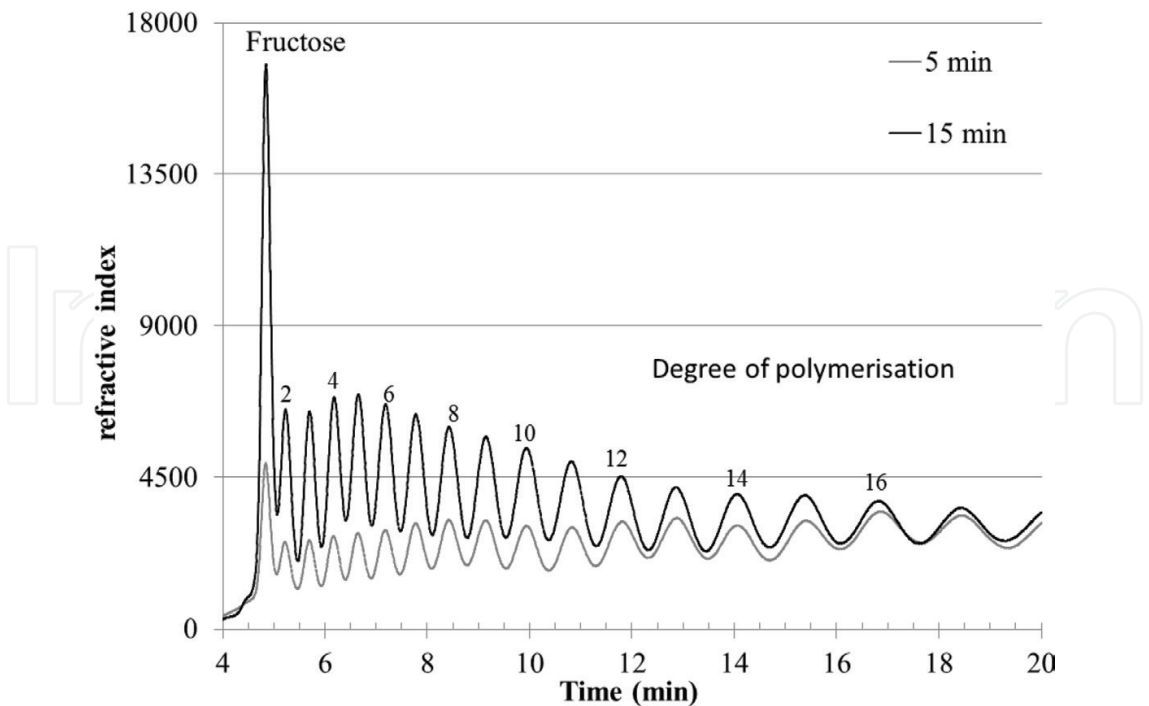


Figure 9. High performance liquid chromatography profile of fructooligosaccharides (FOS) from partial diluted phosphoric acid-catalyzed hydrolysis of inulin. Note: High performance liquid chromatography analysis was performed in a Shimadzu LC-10 apparatus (Tokyo, Japan) consisting of a binary pump and a refractive index detector with a Spectra Amine column (200 × 4.6 mm, 5 μm) (Merck, Germany) and isocratic acetonitrile:water (7:3) at flow rate of 1.0 mL min⁻¹ flow.

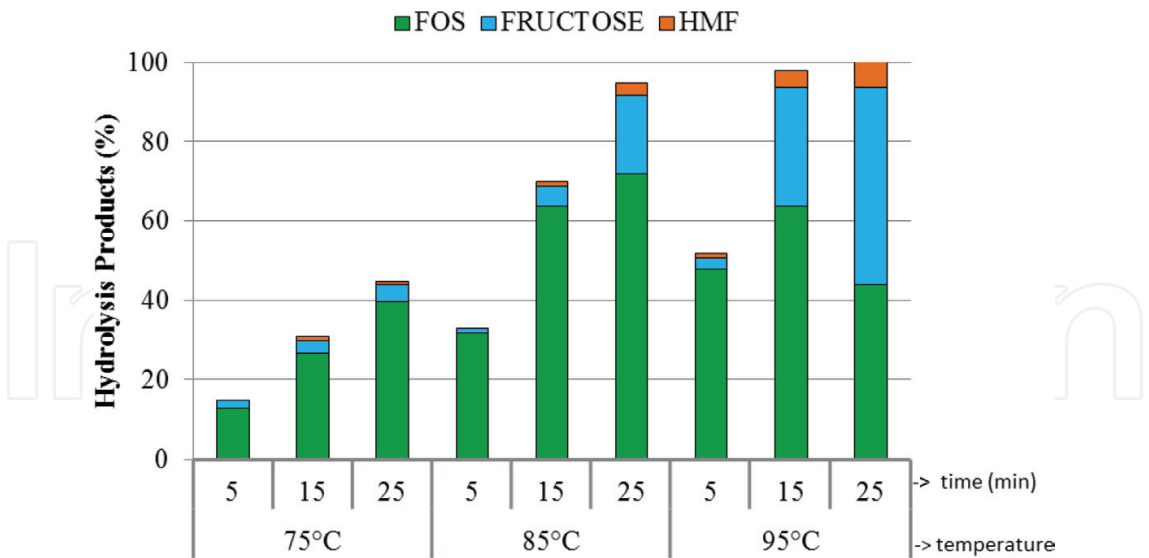


Figure 10. Comparative phosphoric acid-catalyzed production of fructooligosaccharides and fructose from inulin from *Dahlia* tubers inulin, exploring the kinetic variables time of reaction and temperature at a pH of 2.5.

seems to be in discontinuation), amylases still follow as good industrial tools (as is the case of corn starch conversion to glucose, in the USA) [43].

The quantitative distribution of released AMOS (AmyloOligosaccharides with DP from 2 to 10) following the severity parameter of H_3PO_4 mass as 32.4 or 132 mg g^{-1} starch and thermopressurization peak at 159°C (5 atm) were evaluated by densitometric measurement of spots intensity of a TLC plate (revealed with 0.5% orcinol in H_2SO_4 :methanol (1:9); heating at 105°C for 5 min) in a Shimadzu equipment model flying spot CS-9301PC densitometer as shown in the following illustrations (**Figures 11** and **13**).

11.3. Ligno(hemi)cellulosics [L(h)C]

Ligno(hemi)cellulosics [L(h)C(s)] are by far more complex polymeric occurrences in phyto-biomasses. In fact, cotton is a very peculiar natural form of pure cellulose since it is almost completely free of hemicelluloses and lignin (the case of cotton balls and not the whole plant). In average, L(h)Cs such as sugar cane bagasse, corn and other cereal straws, soya hulls, timber sawdust, and related materials are a tightly interbonded native architecture of cellulose:hemicellulose:lignin in an average proportion of 50:30:10 with lesser amounts of pectin, other minor polysaccharide, protein, salts, and organosolvent-soluble extractives.

The two main distinctive features among L(h)Cs are the monolignol units in lignin (coumaryl, syringyl, and guayacyl) and the particular structure of hemicelluloses moieties as heteroxylans in hardwoods and grasses (angiosperms; monocotyledons) and heteromannans in softwoods (gymnosperms), although a minor amount of xylan is also present in conifer trees. The order of decreasing resistance to acid hydrolysis is: cellulose > mannans > xylan. It is also worth mentioning that xylan have other constituents (arabinose and (4-O-methyl)-glucuronic acid) are single branches (besides some O-acetyl groups in part of the basic xylosyl units), while the presence of glucose in conifer mannans is an intercalation between the basic mannobiosyl or mannotriosyl linear units. More, xylan branches are α -glycosidically linked, whereas glucosyl units of mannans are β -linked. In another words, L(h)Cs from hardwoods and grasses (e.g., cana bagasse) may be treated with thermopressurized phosphoric acid with two completely different purposes: (a) depolymerization (complete till free of xylose or partial till XOS – XyloOligoSaccharides); (b) almost complete lignin solubilization in the same phosphoric hydrolysate; and (c) labilization of the native cellulose component toward a new form (probably less crystalline) which is more prone to the action of cellulolytic enzymes.

A first report on phosphoric acid combined to other mineral acids for the depolymerization of oat straw came from the United States Department of Agriculture (USDA) researchers. The bioconversion of the released free sugars to grow less usual yeast, like *Aureobasidium pullulans*, and then using the hydrolysates as feeder for a field rodent [44]. Followed some years later, by our pioneering work with very diluted aqueous and thermopressurized phosphoric acid to monomerize cane and sorghum bagasse, to render the residual cellulose more labile to celluloses and hence its hydrolysis to free glucose to the final bioconversion to (bio) ethanol [45].

Here are plenty of chromatographic illustrations of factorial designs in order to optimize the hemicellulose hydrolyses of polysaccharides; the native models are sorghum starch, wheat hulls, and sugar cane bagasse. These illustrations correspond to **Figure 11**.

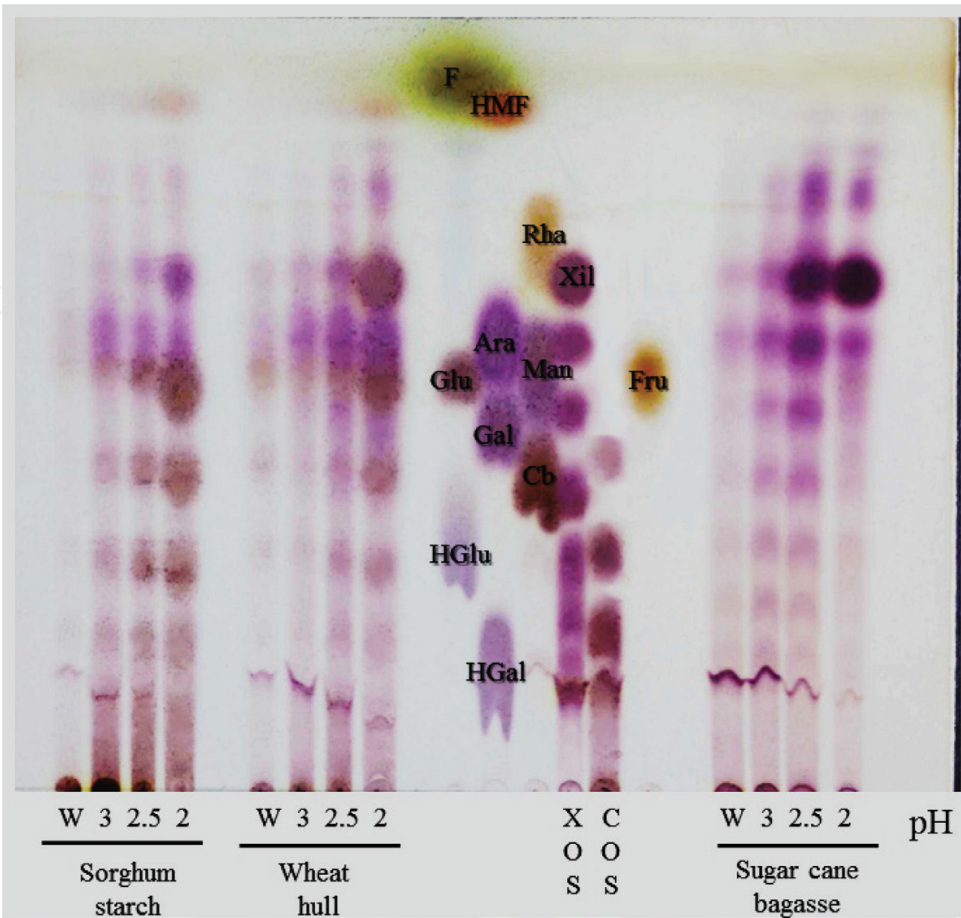


Figure 11. Thin-layer chromatographic for the progressive hydrolytic fragmentation of sorghum starch, wheat hulls, and sugar cane bagasse with pressure of 6.0 atm (165°C) and with water (w) and *o*-phosphoric acid pH of 2.0–3.0. Note: Center: standards of furfural (F), xylose (Xil), glucose (Glu), hydroxymethylfurfural (HMF), arabinose (Ara), rhamnose (Rha), fructose (Fru), galactose (Gal), mannose (Man), cellobiose (Cb), glucuronic acid (HGlu), galacturonic acid (HGal), cello oligosaccharides (COS) and xylooligosaccharides (XOS).

Formerly, FOS and later XOS, MOS, and GOS were reported as nutraceutical oligosaccharides deserving a better industrial and medical exploration. Some polysaccharides such as inulin and resistant starches are designed as functional foods or those who brings to men and animals benefits other than the simple nutrition. Nutraceutical is any food additive that corresponds to a concentrated form of a determined functional food. As a simple example, if carrot is considered a functional food, thanks to its antioxidant and free radicals fighter, β -carotene, its safe organosolvent extract (e.g., hot ethanol) may be considered the respective nutraceutical extract. *Bifidobacterium animalis* and *Lactobacillus casei* are two good examples of colonic beneficial bacterial microflora whose growth can be bioassayed for the appropriateness of nutraceutical oligosaccharides obtained from cane bagasse or pine sawdust. **Figure 12** shows the selected probiotic growth results.

Let us emphasize on the probable advantageous features of phosphoric acid as an ideal catalyst for phytobiomass polysaccharides depolymerization and further bioconversion to biofu-

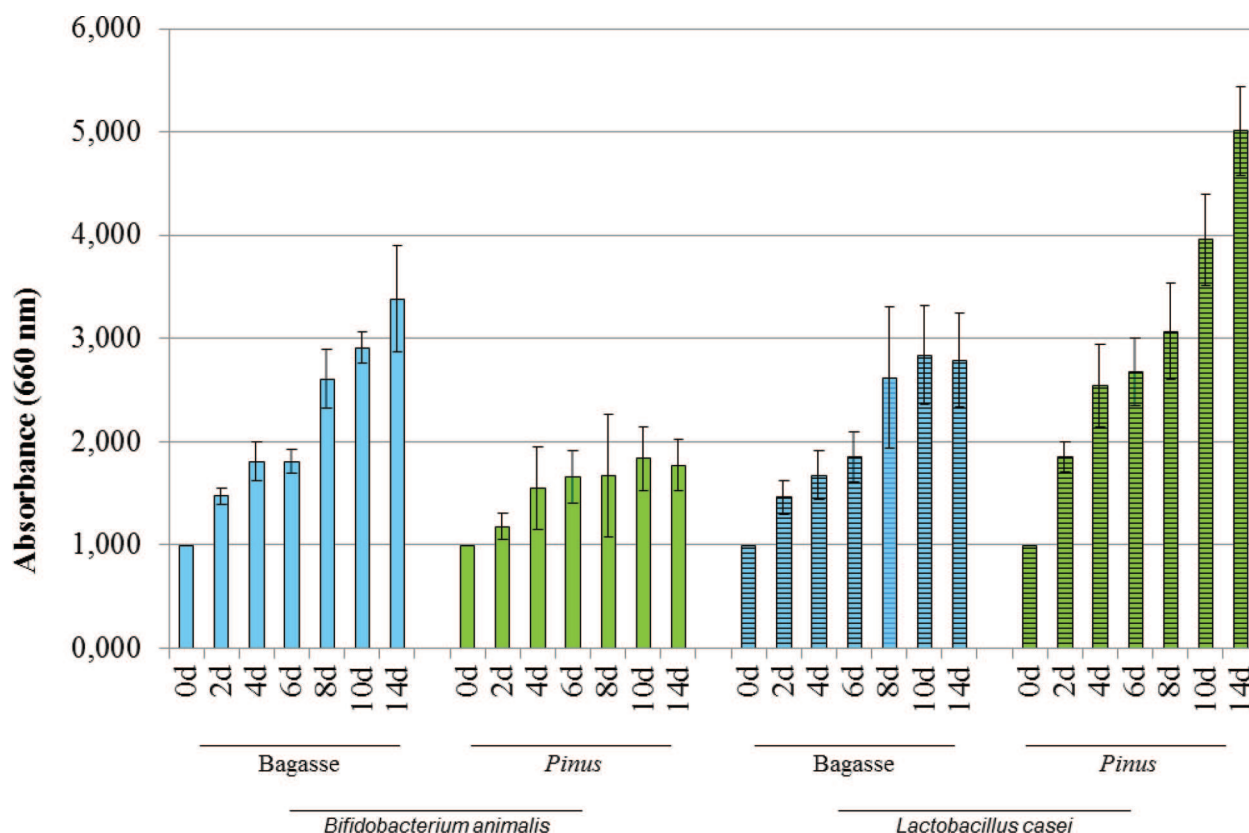


Figure 12. Growth of probiotic *Bifidobacterium* and *Lactobacillus* measured by spectrophotometric analysis on nutraceutical or prebiotic xylo- or mannooligosaccharides.

els and other industrial goods as compared to the stronger mineral acids. The hydrolysates resulting from the latter asks for desalting using expensive resins since chloride residual anions are often inhibitory to yeasts and other useful fermentation of microorganisms. Sulfate, if not so, is also less usefulness despite a minimum amount may be turned to the sulfur aminoacid methionine, although there are many steps of anabolism. Conversely, phosphoric may be maintained in the free sugar or oligosaccharide hydrolysates since together the latter and after partial neutralization with ammonia (or any other desirable base) it composes the usual and mandatory triplet for any industrial fermentation: free sugars and N and P sources.

Figure 13 assembles a comparative performance of diluted heated phosphoric acid, in this case through a simplified procedure, namely, an oven in Teflon-lined lid vials, for the partial (even total as well, if desirable) hydrolysis (oligosaccharidogenic effect) of corn cob xylan, *Dahlia* tubercles inulin and potato starch. All three oligosaccharidic families (OS, FOS, and MaltOS), then just following a partial neutralization till pH 4–5 with ammonia or soda may be used as C source for the growth of colon-beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* spp., thus acting as nutraceutical oligosaccharides or symbiotics if as result of the blend of reagent (prebiotics) and products (probiotics).

Maltosaccharides are important feedstuff for the formulation of infant food. They can be easily produced using *o*-PA-catalyzed starch partial depolymerization (**Figure 14**). From the nutri-

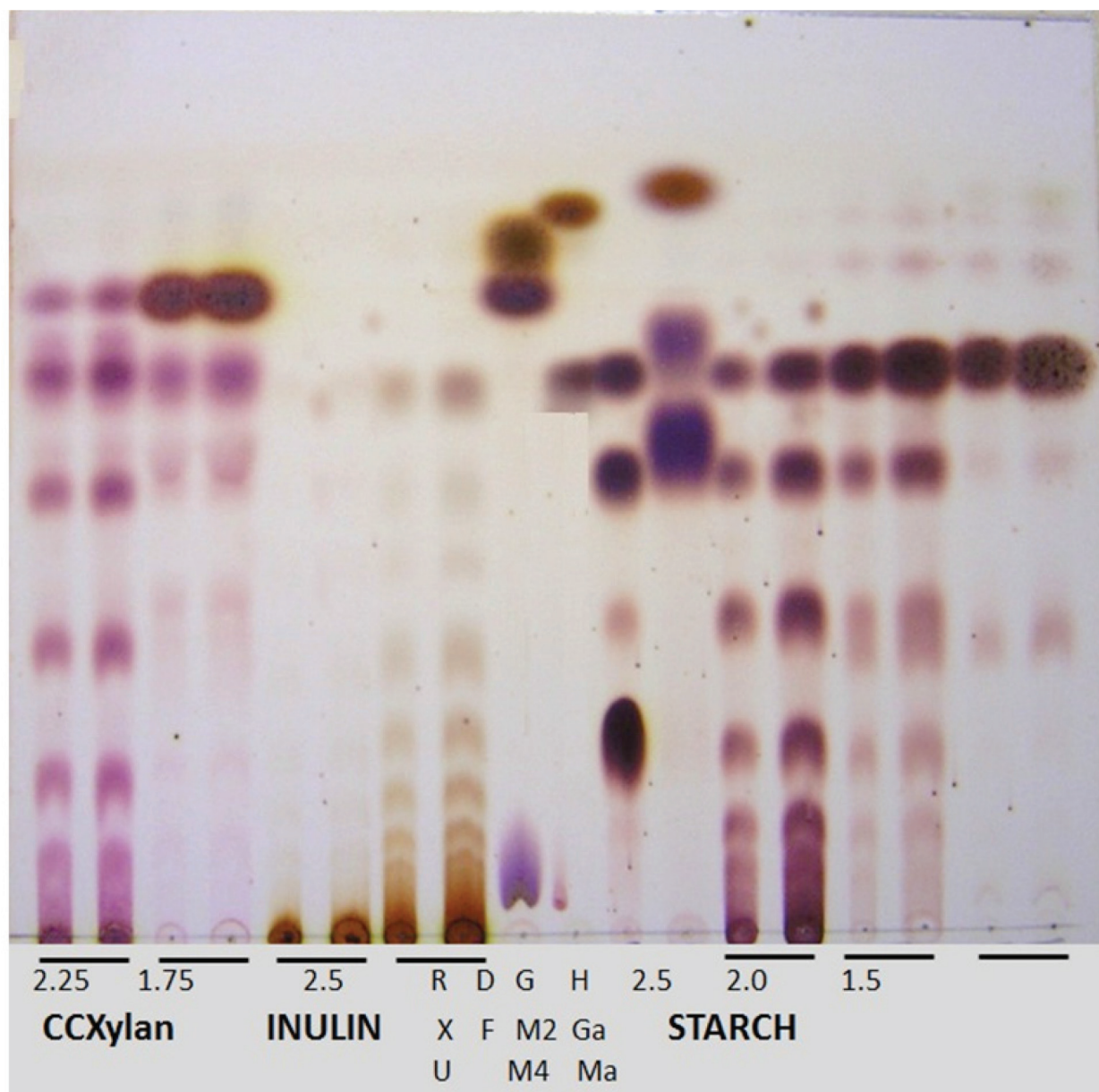


Figure 13. Thin-layer chromatographic of partial heated diluted phosphoric acid hydrolyses of corn cob Xylan, dahlia tubercles Inulin, and potato starch. Note: Numbers = hydrolyses pH at 160°C for 5 min for Xylan and Starch or at 80°C for Inulin (hydrolysates neutralized with CaCO_3); Standard: R, X, U = rhamnose, xylose and glucuronic acid; D, F = difructose III anhydride and fructose; G, M2, M4 = glucose, maltose and maltotetraose (with traces of maltotriose); H, Ga, Ma = hydroxymethylfurfural, galactose and mannose. Eluent = isopropyl alcohol:ethyl acetate:water (7:1:2).

tional standpoint, the neutralized catalyst with ammonia or alkaline bases can add important sources of P and N, for instance.

The destination of total or partial *o*-PA-hydrolyzed polysaccharides, depending on its source and hence carbohydrate composition, may be fermentation to biofuels (bioethanol or

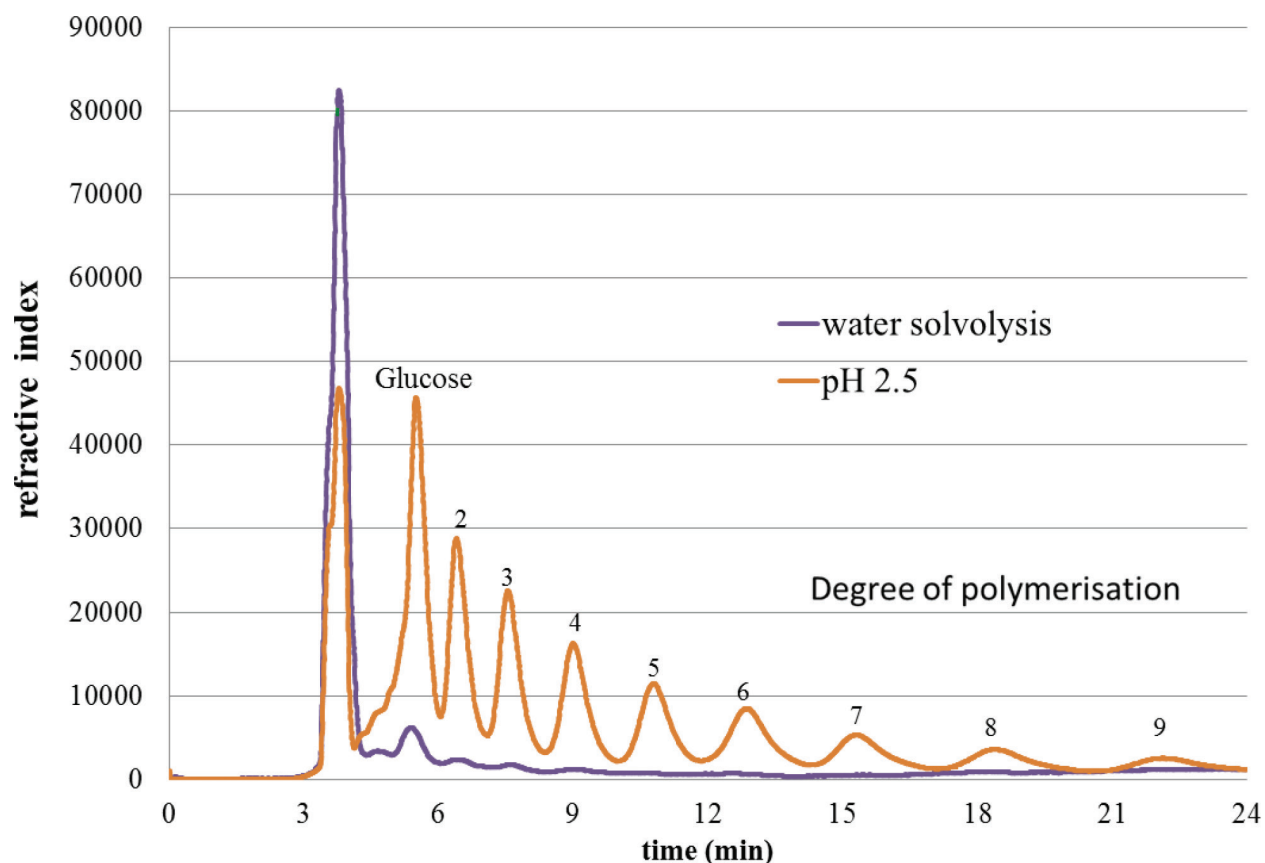


Figure 14. Oligosaccharide profiles of depolymerization of sorghum starch: comparative efficiencies of solvolysis (water) and *o*-PA. Note: 2–9 denotes for maltooligosaccharides with DP from maltose (2) till maltononaose (9). High performance liquid chromatography analysis were performed in a Shimadzu LC-10 apparatus (Tokyo, Japan) consisting of a binary pump and a refractive index detector with a Spectra Amine column (200 × 4.6 mm, 5 μm) (Merck, Germany) and isocratic acetonitrile:water (67:33) pH 9.0 (1.0 mL min⁻¹ flow).

second-generation ethanol with *Saccharomyces cerevisiae* whenever free glucose from starch or fructose from inulin) or alternatively from pentoses and C5-oligosaccharides with other yeast such as the genders *Pichia*, *Candida*, and *Spathaspora* or generation of probiotics biomass (*Lactobacillus* and/or *Bifidobacterium* spp.) or even symbiotics (probiotics + prebiotics such as nutraceutical oligosaccharides) whenever the partial hydrolysates are richer in the appropriated oligosaccharides [46, 47].

For instance, soya husks hydrolyzed with pH 2.5 *o*-PA at 7 atm (171°C) released xylose a xylo-oligosaccharides as the major hydrolytic products, then converted to ethanol by the yeast, a known C-5 sugar ethanologenic fermenter (**Figure 15**).

Another example is the production of short chain fatty acids (SCFAs) by the beneficial enterobacterium *Bifidobacterium* growing in *o*-PA-partially hydrolyzed cell wall glycans from the cyanobacterium *Arthrospira* (formerly, *Spirulina*) (**Figure 16**). SCFAs are responsible for the colonic pH drop in turn inhibiting the growth of harmful coliforms, these implicated in the generation of aberrant crypta and later tumors [48].

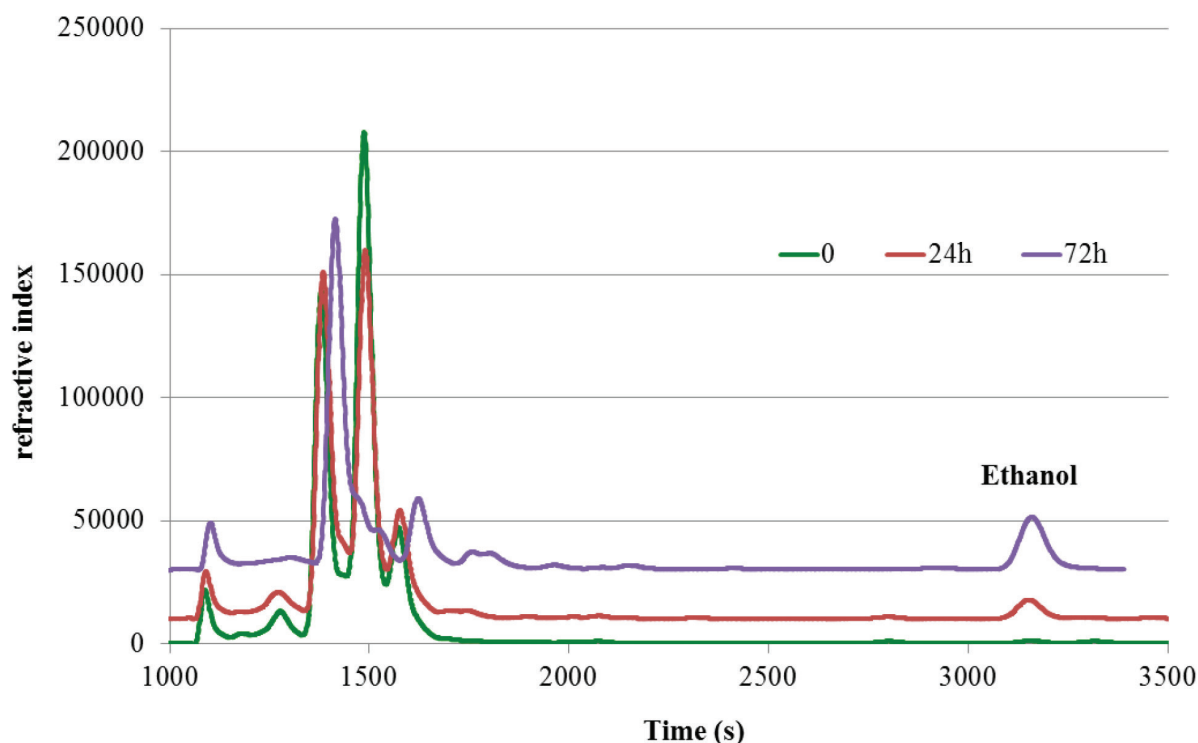


Figure 15. The progressive generation of ethanol from the yeast *Pichia stipitis* growing on *o*-PA-hydrolysed soya husks between 24 and 72 h of culture at 30°C. Note: High performance liquid chromatography analysis were performed in a Shimadzu LC-10 apparatus (Tokyo, Japan) consisting of a binary pump and a refractive index detector with a Rezex ROA column (300 × 7.8 mm, 8 µm) (Phenomenex, USA) and isocratic 8 mmol L⁻¹ H₂SO₄ (0.5 mL min⁻¹ flow).

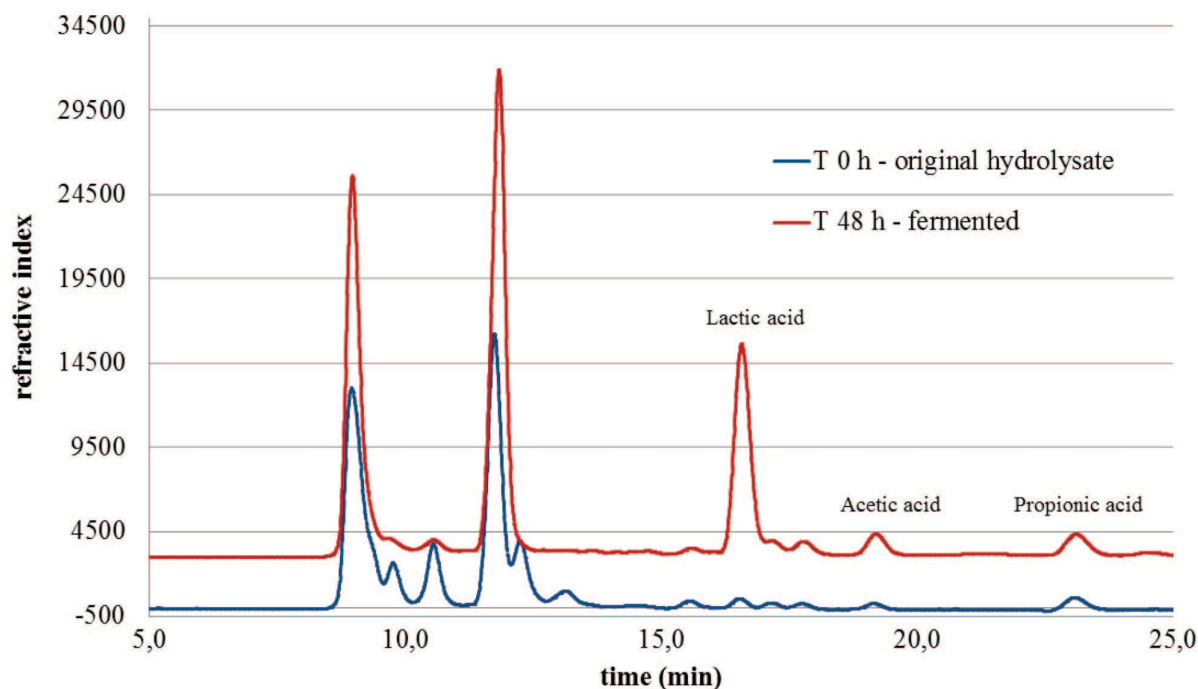


Figure 16. Time course of short chain fatty acids (SCFAs) production from the *o*-PA-hydrolyzed *Arthrospira platensis* (cyanobacterium) cell wall polysaccharides after incubation of the hydrolysate with *Bifidobacterium animalis*. Note: High performance liquid chromatography analysis was performed in a Shimadzu LC-10 apparatus (Tokyo, Japan) consisting of a binary pump and a refractive index detector with a Rezex ROA column (300 × 7.8 mm, 8 µm) (Phenomenex, USA) and isocratic 8 mmol L⁻¹ H₂SO₄ (0.5 mL min⁻¹ flow).

12. Phosphate analytical determination

There are a plenty of analytical methods for phosphate spectrophotometric determination. Examples are: molybdate/hydrazine [49], perchloric acid/molybdate/ascorbic acid (specially on phospholipids) [50], molybdate/Triton X-100 [51, 52], molybdate/quinolone [53], and molybdate/vanadate, too.

A comfortable and precise chromatographic determination is the utilization of ion chromatography that allows the parallel quantitation of other organic acids such as citric acid since both are widely used acidulants in the food and beverage industry [54].

13. Special applications of phosphoric acid and their derivatives

From the last century till recently, many novel technological applications of phosphoric acid came to light. A few examples are:

- [A] An innovative chiral center was built on phosphoric acid by the insertion of two acidic phenolic groups. The compound acted as an efficient catalyst in the enantioselective alkylation of enones and indoles through the Friedel-Crafts methodology [55].
- [B] 3-3-pentadecylphenylphosphoric acid (PDPPA), inserted with a side chain of a long hydrophobic hydrocarbon, as a doping for polyaniline and increasing its solubility in organo-solvents, the plasticity for flexible films and allowing 1.8 S cm^{-1} of high conductivity [56].
- [C] Cyclic ester amides of phosphoric acid (e.g., from N,N-bis-(B-chloroerhyl)-0,0-ethylene) were synthesized and proposed as favorable therapeutic cytostatics in the treatment of tumors [57].
- [D] L-NASPA, N-palmitoyl-L-serine-phosphoric acid found its application as inhibitor of lysophosphatidyl receptors and affecting Ca^{2+} concentration in human sarcoma MG63 cells, thus indicating potential application in therapy [58].

Most of the applications are concerned, more recently, with improved fuel cells.

- [E] In order to increase temperature resistance, a thermostable HT-PEMFC was built on polybenzimidazole previously doped with phosphoric acid. A range of $0.06\text{--}0.12 \text{ S cm}^{-1}$ was achieved [59, 60].
- [F] Fuel cells built on a composite of phosphoric acid and quaternary 1,4-diazabicyclo-[2.2.2]-octane (DABCO) polysulfone. Low and high degree of substitution led to the respective conductivities of 0.064 and 0.12 S cm^{-1} [61].
- [G] Activity of phosphoric acid as a fuel cell electrolyte allowed operation at a higher temperature without loss of conductivity or circuit voltage. The novel membrane is based in the coordination of PO_4 —groups with silicon which allowed power density of 184 mW cm^{-2} at 226°C with an H_2 flow rate of 4.1 mL min^{-1} [62].

[H] The direct oxidation of methanol in full cells (DMFC) was achieved in membranes made by grafting epoxy groups through PGMA, poly(glycidylmethacrylate). Imides were then generated therefrom and synthesis cycle was closed with phosphoric acid doping. The product was said to be of lower cost and with a performance superior to Naphion 117 [63].

14. Advertisement for readers

For those who wish a sharp view on recent advances on phosphoric acid, phosphates, and agricultural aspects of their applications and innovations, the recommendations are the annual series of "SYMPHOS" (International Symposium on Innovation and Technology in the Phosphate Industry) whose usual venue is Marrakesh, Morocco. For instance, the 2015 event offered "Jacobs® New Process for Removing Iron from Phosphoric Acid" [64]; "Thickening, filtration and clarifying in Phosphoric Acid industry" [65]; "Comparison of different ways of desulfatation used in OCP phosphoric acid plants," (H. Mourchid, Responsible of Phosphoric Production, Maroc Phosphore Safi, OCP S.A., Morocco).

The 2013 edition of a review also encompasses a deeper view of advances experienced in both research and application of phosphates [66].

Beyond the scope of this chapter is the mention of worldwide generation of patent request in the subject, an interest which may be satisfied though quick searches on the recommended sites such as USPTO (USA), EPO (European Patent Office), and so on, all of them recorded at World Intellectual Property Organization (WIPO) and its directory.

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